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A RE-EXAMINATION OF DIPLECTRONA MODESTA BANKS 1908 (TRICHOPTERA: HYDROPSYCHIDAE) USING MORPHOLOGICAL AND MOLECULAR TECHNIQUES

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A RE-EXAMINATION OF *DIPLECTRONA MODESTA* BANKS 1908 (TRICHOPTERA: HYDROPSYCHIDAE) USING MOLECULAR MORPHOLOGICAL AND TECHNIQUES

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ABSTRACT

Diplectrona modesta Banks 1908 (Trichoptera: Hydropsychidae) is a common inhabitant of streams across eastern North America. This species is well known and used often in biomonitoring studies. Experts in the field have long noted the morphological variation in larval head color and patterns as well as the species presence in many different types of habitats, such as springs, seeps, streams, and some small rivers. This study examines the species both morphologically and molecularly using mitochondrial and nuclear genes in order to determine if the species is a complex of several species or one highly variable species. DNA sequencing using the mitochondrial COI gene and D2 region of the 28s nuclear gene revealed 5 haplotypes, indicating a complex of several species or morphs. Morphological variations of the larval head capsule correspond with the haplotype grouping. No corresponding traits were found in the adults of the species. Without characters to separate the adults along with the larvae, these haplotype groups will not be described as new species at this time.

DEDICATION

I’d like to thank my friends and family for their support over the last few years. Their understanding and encouragement have helped me push through difficult times and reminded me how to celebrate the wonder of science, nature, and education in this world. My parents introduced me to nature at a young age and I still remember spending days looking under rocks with my father and learning the names of wildflowers with my mother. Those early years left me with a strong passion for all things natural in this world and I thank them for passing that on to me and my brothers. In addition to my parents, my brothers, grandparents, cousins, and aunts and uncles have gone out of their way to understand my project and help whenever possible, and that help has brought us closer and reaffirmed that family is the most important part of life.

This thesis is dedicated to all of them for their unconditional love and support.

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First and foremost, I'd like to thank my fellow labmates and students for the unlimited help and support on this project. Former graduate students C. J. Geraci (Smithsonian Institution and National Science Foundation), I. C. Stocks (Florida State Collection of Arthropods), M. J. Biondi (Smithsonian Institution and National Science Foundation), and J. Robinson (University of Tennessee), have all contributed to this study by providing specimens, advice, or both. Several of our projects had overlapping themes and by working together we were able to amass a great amount of data that helped us all.

I spent several months in New Jersey at Rutgers University working with Dr. Karl Kjer and Dr. Geraci who taught me how to extract and amplify DNA, as well as align and analyze the results. I learned everything at the lab there and could never articulate my gratitude to those people who saved me when I was completely "lost" in the world of genetics. I'd also like to thank the Kjer family for their hospitality while I stayed with them.

My advisors, Dr. P.H. Adler and Dr. W.R. English (both at Clemson University) also played important roles in my time at Clemson. Classes were my favorite part of being back in school and their classes in particular were inspiring and reminded me every day of why I loved this field. I am very grateful to them all for guiding me through this process.

My advisor, Dr. John Morse, not only introduced me to caddisflies but has since given me great insight into their beauty, unique life history, and ecological importance. He helped me design this project and guide my research and writing processes. His patience has meant the world to me and I hope he can be proud of this work.

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INTRODUCTION

General *Diplectrona* Overview

The order Trichoptera consists of caddisflies, a group of insects that spend their larval phases in aquatic environments. This group is holometabolous and one of the most diverse groups of insects in the world due to striking diversity of habitats used and exploitation of food resources. This order is divided into three suborders, monophyletic Annulipalpia and Integripalpia, and probably paraphyletic “Spicipalpia” (Morse 1997). The monophyletic family Hydropsychidae (Geraci 2010) is in Annulipalpia (Merritt, Cummins & Berg 2008). Larvae of Annulipalpia form retreats from small cobble, plant matter, and silk. They are not confined to cases as are Integripalpia and some “Spicipalpia,” or free living, like others of “Spicipalpia” (Triplehorn & Johnson 2005).

Larvae of the “net-spinning caddisflies” (family Hydropsychidae) spin labial silk into retreats and nets, which are oriented in cool, lotic habitats to catch fine particulate organic matter (FPOM) for food (Triplehorn & Johnson 2005). These collectors-filterers sieve FPOM with their nets which are spun with an intermediate to large mesh size (Wallace 1976 a,b). Most species are univoltine, completing a life cycle in one year, with larvae maturing through 5 instars before metamorphosis. The larvae of Hydropsychidae can be separated morphologically from other families by the long setae arrayed in a fan above the apical hook on the anal proleg, a completely sclerotized metanotum, and branched gills that run ventrolaterally along the abdomen (Triplehorn & Johnson 2005). The pupae attach themselves to the bottoms of rocks large enough to be stable.

Adult hydropsychids are characterized by a lack of both mesoscutal warts and ocelli and the presence of 5-segmented maxillary palps, among which the last segment is long and has fragmentary or "annulated" sclerotization (Triplehorn & Johnson 2005). Adults emerge during the summer months and in general live about a month, during which they mate and lay their eggs.

The hydropsychid subfamily Diplectroninae in North America includes the type genus, *Diplectrona* (Westwood 1840), *Homoplectra* (Ross 1938), and *Oropsyche* (Ross 1941). *Homoplectra* larvae differ from those of *Diplectrona* by the position of the transverse furrow on the pronotum. The adults of *Homoplectra* have less broadly rounded hind wings and the front wings have crossveins m3+4-cu1 and cu1- cu2 less closely situated than in the hind wings. The Sc and R1 veins of the front wings are also more strongly curved in *Diplectrona* than in *Homoplectra*. *Oropsyche* larvae are unknown and adults exhibit apical incisions on both sets of wings (Ross 1944). Adult specimens of *Oropsyche* have been collected only a few times and are believed to be day-flying, unlike most other caddisflies.

The genus *Diplectrona* can be found in all zoographic regions except the Afrotropical, Neotropical, and Antarctic regions, with egg masses, larvae, and pupae usually found in cool, rapid running water of springs, small streams, and small rivers (Wiggins 1996), and with adults flying and resting in nearby riparian habitats. There are 5 species known from the,Nearctic region, including *Diplectrona californica* Banks, 1914; *D. marianae* Reeves, 1999 (in Reeves & Paysen 1999); *D. modesta* Banks, 1908; *D. metaqui* Ross, 1970; and *D. rossi* Morse, 1990 (in Morse & Barr 1990). In North America, *D. californica* has been recorded only in the state of California, *D. marianae* has been recorded only in a cave system in Georgia, and *D. rossi* has been recorded only in Louisiana. *Diplectrona modesta* and *D. metaqui* have much larger distributions. *Diplectrona modesta* is widespread across the middle to eastern regions of the United States and Canada from Minnesota, Ontario, and Newfoundland in the North; to South Dakota, Oklahoma, and Texas in the Midwest; to Florida in the Southeast. *Diplectrona metaqui* has been recorded in the following eastern states: Connecticut, Georgia, Illinois, Indiana, Kentucky, Missouri, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, and Virginia.

The larva of *D. californica* has not been described, but larvae of the other 4 North American species are known. Larval *Diplectrona* species are usually distinguished from each other by head and body color patterns, the shapes of the mandibles and their teeth, and the positions of setae on the body. The larva of *D. metaqui* has a thumb-like process projecting from the dorsal edge of the left mandible whereas *D. modesta* does not (Ross 1970). *Diplectrona marianae* is a sister species to *D. metaqui* and the larvae feature a similarly notched frontoclypeal apotome (Reeves 1999). These 2 species can be distinguished from each other, however, by the ridge-like process on the left mandible of *D. marianae* which is less pronounced than the thumb-like process of *D. metaqui* (Reeves 1999). The notched frontoclypeal apotome of *Diplectrona marianae* distinguishes it from *D. rossi* (Reeves 1999). *Diplectrona rossi* is diagnosed by 3 regions of red-yellow color in the lateral and posterior regions of the larval frontoclypeus (Morse & Barr 1990).

The genitalia of adult *Diplectrona* species are generally similar, with only subtle differences. However, other morphological characters such as eye size and placement, body and antennal color patterns, and head wart size can be used to diagnose species from adult specimens (Ross 1944). For example, adults of *Diplectrona metaqui* can be differentiated from those of *D. modesta* by smaller middle head warts in both sexes and smaller eyes in adult males in *D. metaqui* (Ross 1970). *Diplectrona marianae* adults can be distinguished from those of *D. californica* by the lack of dorsal lobes on the male tergum X (Reeves & Payson 1999). *Diplectrona marianae*'s lack of a pair of truncate dorsolateral lobes on male tergum X distinguishes it also from *D. rossi*, while the anterior wart of the head of *D. marianae* is larger and more rounded than that of *D. modesta* (Reeves & Paysen 1999). *Diplectrona rossi* is different from *D. metaqui* and *D. modesta* because the width of the adult head lies between the widths of the others (Morse & Barr 1990). Adults of *D. rossi* have smaller eyes than *D. modesta* and larger eyes than *D. metaqui* and the male of *D. rossi* has a more truncate apex of the dorsolateral lobe of abdominal segment X (Morse & Barr 1990).

Diplectrona modesta sensu lato is a common inhabitant of headwater streams across most of eastern North America (Nimmo 1987, Morse 2010). It is found at many elevations, from the mountains to the coastal plain, and in cold springs with barely any water flow to small rivers (Gordon & Wallace 1975). In general, the highest concentrations of *D. modesta* within a watershed will be in the headwater streams, mid to large creeks, and the small rivers that have the appropriate oxygen content, food sources, and substrate. They build their retreats in crevices on the underside of rocks and stones, probably to avoid predation (Merritt *et al*. 2008). Pupae also build their pupal cases on the underside of stones (Karl & Hilsenhoff 1979). The larvae are omnivores, feeding primarily on vegetation and animal parts they filter from the water in silken nets (Wiggins 1996). The larvae spin the nets with salivary or labial glands that produce silken threads (Wiggins 1996). These glands are similar to those found in the order Lepidoptera, Trichoptera's closest relative (Wiggins & Wichard 1989). The nets are oriented upstream to catch the particulate matter that flows in the current (Merritt *et al*. 2008).

Headwater streams are considered isolated environments because the mountains that surround them usually form barriers to dispersal. The variation among *D. modesta* larvae individuals in body size and color of the head capsule, combined with the wide distribution and isolated habitats, has prompted the hypothesis that several similar species exist (Morse & Barr

1990). The morphological variations are subtle and to split *D. modesta* into several species based on these characters alone may not reflect actual species bounds, yet the variations are perplexing. It is also unusual for a Trichoptera species to have so much variation in the larval form without having at least as much variation in the adult form. Most caddisfly species are distinguished by the genitalia of the adults whereas the larval forms are ordinarily more difficult to identify to species. To diagnose *D. modesta* confidently, or to declare that the morphological variations are present because *D. modesta* is a single highly variable species, required some molecular studies of the genome.

According to Graur & Li (2000), using molecular techniques to differentiate species can be more useful than using morphological techniques alone. Morphological characteristics can be affected by the environment and therefore may not sort congruently, whereas molecular characteristics are heritable, not affected by the environment, and easier to define. According to these authors, molecular traits evolve more regularly and give a clearer picture of relationships through quantifiable methods than do morphological traits. Finally, molecular data are more abundant than morphological data (Graur & Li 2000). These advantages may make molecular work worthwhile. Nevertheless, to conduct a comprehensive study and to facilitate routine identifications, one must still consider morphological data and attempt to discover any correlation between the 2 types of data.

An intrapecific study requires comparisons of rapidly evolving genes. More slowly evolving genes that, for example, may show variation only at the family and more-inclusive taxonomic categories would be inappropriate to show sequence differences among species and conversely, more rapidly evolving genes may show variations between related individuals that would be too variable to use for species differentiation. Mitochondrial DNA has been used the past to delineate species complexes in insect groups (Bogdanovic *et al*, 1993, Frey *et al*. 1995, Sperling *et al*. 1995, Brower 1999,Landry *et al*, 1999, Kruse *et al*.2001). Previous molecular studies in animals have successfully focused on the mitochondrial gene cytochrome c oxidase subunit 1 (COI) to show differences in the genome at the genus and species levels (Herbert *et al*. 2004). Mitochondrial genes have a high copy number and are easy to amplify, which make them primary candidates for DNA barcoding. The successful use of COI in lepidopteran investigations lends confindence to a similar approach when working with Trichoptera (Herbert *et al*. 2004). In this particular case, the COI gene is also convenient because the primer sequences for it in Trichoptera have already been established by Dr. Karl Kjer at Rutgers University.

In addition to COI sequencing, there is a growing interest in using nuclear ribosomal sequences from genes such as the D2 region of 28S to look at species differences. In the past, nuclear genomes have been used for higher level studies but recently some investigators have found that the D2 sequence results correlate well with COI results (Zhou, Kjer & Morse 2007). This lends an extra level of confidence when determining the relatedness of a species group.

Objective

To determine whether *Diplectrona modesta* is one variable species or comprises a species complex.

Hypothesis

H_O: *Diplectrona modesta* is a single morphologically variable species.

H_A: *Diplectrona modesta* is a complex of more than one species.

Predictions

Prediction 1: No distinct haplotypes of mtCOI or D2 will be found with a branch length of more than 2%.

Prediction 2: If haplotypes of mtCOI and D2 are found, they will not be congruent.

Prediction3: If haplotypes of mtCOI and D2 are found and they are congruent, these haplotypes will not be congruent with larval morphotypes.

MATERIALS AND METHODS

Morphological Techniques

Collections. The author and several volunteers acquired fresh, fixed specimens of *Diplectrona modesta*. The states of Ohio, West Virginia, Kentucky, Tennessee, Virginia, Georgia, South Carolina, and North Carolina were all visited for collections. (Table 1).

General Observations. *Diplectrona* larvae were collected along the Appalachian Mountains. Focus was given to springs, seeps, and first and second order streams, but creeks and rivers were also investigated. Collections were made over a 2-year period (2005-2006), with of the samples used for the study collected during the second year. Larvae were found in their retreats, usually consisting of small cobble and vegetation on the sides and bottoms of larger stones in the stream bed. In the small springs, the larvae were found at the source with numbers dwindling with increased distance from the source. In each location, larvae of *Diplectrona* and larvae that resembled *Diplectrona* were collected during the day; at night, light traps were set to collect adults. The light traps were positioned in and around the area where the larvae were collected that day and were operated from approximately 20 minutes before sunset until around midnight. Occasionally Light trap samples were sorted the next morning and stored in 100% ethanol until examination in the lab.

Diplectrona dominated the caddisfly fauna in the springs and seeps that were visited. Larvae were in first order streams and were found in areas dominated by other hydropsychids. During the summer of 2005, drought affected many of the perennial streams in collecting areas, but *Diplectrona* larvae were found when no other caddisflies were present.

Physical and chemical conditions of collection sites were not recorded, however in the future measures of conductivity would be a priority.

Specimens were also acquired through various state regulatory agencies in the East and several institutions, including, but not limited to the Smithsonian's United States National Museum of Natural History (NMNH) in Washington D.C. and the Clemson University Arthropod Collection (CUAC) in Clemson, South Carolina. The latter currently houses 6,143 *D. modesta* specimens. These specimens were studied under a microscope and both internal and external morphological characters were isolated and used to distinguish individuals within *D. modesta sensu lato*.

Images. Specimens were examined using a Wild M5® dissecting microscope and Dyonics fiber-optic light box at Clemson University. The characters were recorded and described as they were examined. Most adult and larval specimens, whether stored in alcohol or pinned, were also examined and photographed at the Smithsonian Institution, in collaboration with Mr. Jason Robinson (University of Tennessee) and Dr. Christy Jo Geraci (Smithsonian Institution), using a GTEntoVision extended focus photography system and the software Cartograph and Archimed to view the variations more accurately. Photographs of the larvae were taken of the dorsal, left lateral, and ventral views of the head and the left lateral views of the rest of the body, thorax to abdomen. For the adults, photographs were taken of left and/or right lateral views of the head and abdomen based on specimen condition, as well as dorsal views of the head. The photographs of the specimens chosen to represent the variations are used in this paper to illustrate the diagnostic details of the probable species.

Molecular Techniques

Sample preservation. The specimens that were used for molecular analysis were fixed in 100% ethanol in vials and stored at cool temperatures until they were analyzed.

Lab techniques. For larvae and adults, a left middle leg was used for DNA extraction, and the remainder of the specimen was preserved as a voucher to be housed in the Clemson University (CUAC) and the Smithsonian Institution (NMNH). The DNA extraction was conducted using standard Qiagen DNeasy Extraction kits (Qiagen, Hilden, Germany). Once isolated, the DNA was PCR-amplified with primers, dNTPs, and *Taq* polymerase (Hacket *et al*. 2000). PCR products underwent electrophoresis on agarose gels, were stained with ethidium bromide, and were examined under UV light. If the DNA was not isolated and amplified cleanly, low-melt agarose gels were used for electrophoresis. Once clean DNA was available, it was treated using Big Dye Reagent and sequenced for analysis. The primer sequences and lab techniques described by Kjer *et al*. (2001) served as a reference for all molecular work, which was completed under his supervision at Rutgers University in New Brunswick, NJ. The lab techniques were learned under the direction of Dr. Kjer and Dr. C.J. Geraci, and with guidance from Dr. Xin Zhou at the University of Guelph in Canada.

The mitochondrial COI gene for 16 specimens was successfully sequenced, while the variable D2 region of 28S was successfully sequenced for 20 specimens. Some of the specimens were successfully sequenced for only 1 of the genes, while others overlapped and provided information for both genes. Other sequences of both COI and D2 were added to this data set from the Barcode of Life project and other completed studies involving *Diplectrona* specimens.

Sequence Alignment. Once sequences were obtained from the Clemson University sequencing lab, the files were loaded into LaserGene (v.6; DNASTAR, Inc, Madison, WI) where they were aligned by hand. The 2 contigs sequenced were prepared, aligned, and recorded for comparison in the program.

Sequence Analysis. The sequences were then loaded into the Mesquite program using a Neighbor-Joining analysis that showed the similarities between the specimens collected. The analysis was not meant to show phylogenetic relationships but focused on the overall similarity of sequences in the *Diplectrona* complex.

Voucher specimens sequenced in this research are deposited in the Clemson University Arthropod Collection (CUAC). Voucher specimens sequenced by others are deposited in the institutions cited in BOLD.

RESULTS

Molecular results

Successful acquisition of sequences from the specimens was sporadic. In spite of consistent preparations, extractions, and PCR conditions, sequence collection of both COI and D2 took several attempts for some samples and not all collected specimens were successfully sequenced or included in the molecular analysis.

Sequences obtained were compared in individual COI and D2 analyses as well as in a combined analysis. In all instances, analyses were observed using Neighbor-Joining as a means to compare the similarity of the samples. Results from both the individual and combined analyses revealed 7 haplotype groups within the mix of *Diplectrona modesta* and *D. metaqui* samples. Neighbor-joining trees were used to analyze both the author's data (Tree 2) and that of all the sequences that have been uploaded into BOLD (Tree1). The author's tree (Tree 2) does not include *Diplectrona metaqui* (haplotypes 3 and 4), nor does it show all of the other haplotypes that were present when combined with the BOLD sequences (Tree 2). Tree 2 gives a much more conclusive look at the *Diplectrona* sequences that were collected in areas beyond those collected by the author.

Morphological results

Adults and larvae from all collections were examined and compared in an attempt to find characteristics that would differentiate among the haplotypes. Adult specimens had no characters that corresponded with the haplotypes. The search for diagnostic larval characteristics was more successful, with differences being found in head patterns corresponding with haplotypes. However, no other distinct morphological characters were encountered.

Larvae of haplotype group 1 (= "Species A") are characterized by a dorsal light patch at the apex of the epicranial suture that extends anterad toward the frontoclypeus in 2 circular patches. There are also distinct, circular, light-colored patches at the anterior ends of the frontal sutures, giving the head a light, heart-shaped color pattern (Fig. 1).

Larvae of haplotype 2 (= Species "B") feature similar dorsal light patches, one at the posterior apex of the epicranial suture and another just anterior of it, before reaching the frontoclypeus. However, these patches are considerably less bright and the anterior patch has a distinct circular outline of pigment (Fig. 2).

The larva of Haplotype 3 cannot be described morphologically because its sequences were acquired only from adult specimens and at this point there are no differentiating characters known for the adults of the larval haplotype specimen groups. However, Haplotype 3 grouped very closely to Haplotype 4 in the Neighbor-joining tree. Haplotype 4 is *D. metaqui* which is known for the thumb-like process on the dorsal edge of the left mandible, differentiating it from *D. modesta*. This species was included as an outgroup species to provide a comparison for the *D. modesta* haplotype results (Fig. 3). The close grouping of these 2 haplotypes (Tree 1) suggests that they are both D. metaqui or another complex in its own right.

Haplotype 5 (= Species "D"), like group 3, was sequenced using an adult specimen and thus has no larval description.

Haplotype 6 (= Species “E”) has some very light patches similar to those described in haplotypes 1 and 2 but but can be differentiated by a head pattern consisting of muscle scars that speckle both the dorsal head capsule and the ventral portions of the genae (Fig. 4).

Haplotype 7 (= Species “F”), the most distinct of the groups, can be identified by the dark head with no pattern. The body is comparatively dark, as well, especially the sclerotized regions of the thorax. It also has a bright, nearly white circle around each eye (Fig. 5).

Aside from haplotype 7 and the unknown morphologies of the larvae of haplotypes 3 and 5, the other haplotypes look very much alike, featuring the characteristic orange-colored head and thoracic sclerites and the light lateral coloration of the body.

DISCUSSION

Conclusion: The null hypothesis (H_O) was not supported. Instead the predictions were falsified and the alternative hypothesis (H_A) was corroborated.

The results of this investigation show that the now known *Diplectrona modesta* most likely consists of more than 1 species, perhaps 5 species. Whether these haplotypes should be described as separate species is debatable. Many believe (Hebert et *al*. 2003) that genetic sequence data is enough evidence with which to describe species, whether or not morphological data have been recorded. Others (Brower 1999) question the definitions of the term species and eschew validating species that can be diagnosed with only an expensive, time-consuming method.

In the field of entomology describing species based solely on genetic data is still uncommon. While the molecular data in this case may provide strong evidence for distinct species, the lack of correlated morphological evidence for larval and adult discriminations makes species validation impractical. In some cases, such as that of *Oropsyche howellae* (Ross, 1941), a species was described based solely on 1 adult male; however, the specimen was clearly different from anything else described at the time, prompting a conclusion that it is a valid genus and species. In the case of *Diplectrona modesta*, where the species complex contains nearly identical adults that cannot now be differentiated morphologically from one another, it is more difficult to justify species distinctions.

One of the issues involves the ICZN rules for naming species. There is no way to tell which haplotype of the group corresponds with the holotype of the species complex. The original specimens of *D. modesta* are not in a condition (due to preservation practices at the time of description) that will allow for a molecular analysis to discover its haplotype group. This means that naming the haplotypes as new species without knowing which is the true *D. modesta* could result in synonymy. There was, however, a pattern in collecting sites that showed haplotype 1 was collected farther north that some of the other haplotypes. The type locality of D. modesta is Riverside, MA (Banks 1908) which is now a very urban area. Chances are slim of collecting lectotypes there now but if haplotype 1 is the only group found in the New England region, it may suggest that it may be the most likely example of the original specimen.

There also are several reasons to recognize several valid species. For example, ecological variation in the species complex was great, further suggesting that the complex comprises several species. The larvae were collected year round and adults were collected with light traps from May to August. Collections were made throughout the Appalachian Mountains within many state parks, national forests, and on some private land. In some areas the water flow in perennial streams was so little that specimens were present only in areas under stones where they were protected from desiccation. In other sites the small rivers had strong currents or the water was stagnant in pools that had been isolated from the low water flow. In all cases, the larvae found were residing in their retreats made of large sand grains, small cobble, vegetative material, and/or in the interstitial space between large rocks. The elevation varied greatly, with the species being found from the mountains of GA, NC, WV, and VA to the coastal plains of SC and FL. Observing the variation in habitat alone is enough to make one wonder how a single species could be so adaptable to different habitats, especially when one considers the different food resources and water chemistry encountered in such varied sites.

Furthermore, there was an instance in which more than one haplotype group was found in the same location and habitat. In the year-round collections from Baldwin Creek in Oconnee, County, SC both haplotypes 2 and 6 were found in the same sampling site. This overlap in habitat suggests that there is no gene flow between these 2 populations, which is another sign of speciation. Further study of what resources these 2 groups may be sharing or dominating would add additional information to the question of the closeness of these 2 groups.

Caddisflies play an important role in biomonitoring and water quality studies because of the varying tolerances to pollution among the species and their overwhelming presence in almost all aquatic systems. Biomonitoring with macroinvertebrates has become more and more common since it was first implemented and is slowly finding its way around the globe as an important measure of water quality (Hauer & Lamberti 1996, Morse *et al*. 2007). In many parts of the world, programs can identify specimens only to the family- or genus-level as a result of lack of knowledge about the species present. Taxonomic studies are often seen as a separate field from sampling and identifying collections for biomonitoring purposes. However, the description of species and studies of their natural history and relative sensitivity to changes in habitat lend valuable information to the analyses of water quality. The family Hydropsychidae provides an excellent example of why genus and species identifications are so important. The members of this family show a wide range of tolerances; from highly sensitive to extremely tolerant (Resh & Unzicker 1975, Hauer & Lamberti 1996) and lumping them together in water quality studies could potentially skew the results. It is extremely important that taxonomists and those involved with biomonitoring programs work together to insure accurate evaluations of our aquatic habitats.

Although this study did not validate any new species, it provides another example of the value in combining morphological and molecular studies to discover the true nature of a species complex. In this particular case, further examination of the morphology of the specimens falling into the different haplotype groups could provide enough data for validating multiple species with diagnostic adult features and correlate the species with particular ecological conditions. The *D. modesta* species complex is important to water quality monitoring across eastern North America and, if it is found to include more than 1 species, this new information would not only increase our knowledge about our planet's species diversity, but also will help improve understanding of our precious aquatic ecosystems.

APPENDICES

Table 1. ***Diplectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
GA	35.52066N, 83.41895W	27 May 2006	L. Harvey		Stream Sample	X Larva			
GA	35.52066N, 83.41895W	27 May 2006	L. Harvey		Light Trap				
GA	34.67222N, 83.97639W	14 Jun 2006	L. Harvey	GA6	Stream Sample	X larva			
GA	34.74810N, 84.06438W	14 Jun 2006	L. Harvey	GA7	Stream Sample	X larva			
GA	34.75859N, 84.06643W	14 Jun 2006	L. Harvey	GA8	Stream Sample	X larva			
GA	34.76381N, 84.06686W	14 Jun 2006	L. Harvey	GA3	Stream Sample	X larva			
GA	34.76381N, 84.06686W	14 Jun 2006	L. Harvey	GA3	Light Trap				
GA	34.76390N, 84.06845W	14 Jun 2006	L. Harvey	GA5	Stream Sample	X larva			
GA	34.76390N, 84.06845W	14 Jun 2006	L. Harvey	GA5	Light Trap				

Table 1 (Continued). ***Dipectrona.*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
GA	?	15 Jun 2006	L. Harvey	GA2	Stream Sample	X larva			
GA	34.80096N, 84.61642W	15 Jun 2006	L. Harvey	GA1	Stream Sample	X larva			
GA	34.96322N, 84.62845W	15 Jun 2006	L. Harvey	GA1	Stream Sample	X larva			
GA	34.96322N, 84.62845W	15 Jun 2006	L. Harvey	GA1	Stream Sample	X larva			
GA	34.96322N, 84.62845W	15 Jun 2006	L. Harvey	GA1	Light Trap				
GA	34.96275N, 84.63890W	15 Jun 2006	L. Harvey	GA4	Stream Sample	X larva			
GA	34.96275N, 84.63890W	15 Jun 2006	L. Harvey	GA4	Light Trap				
WV	37.53342N, 81.00130W	9 Jul 2006	L. Harvey		Stream Sample	X Larva			
WV	37.53342N, 81.00130W	9 Jul 2006	L. Harvey		Light Trap				

Table 1 (Continued). *Dipectrona* collected, examined, and sequenced.

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
WV	37.98238N, 80.94021W	10 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	37.98238N, 80.94021W	10 Jul 2006	L. Harvey		Light Trap				
WV	38.00338N, 80.94956W	11 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.30036N, 80.50941W	11 Jul 2006	L. Harvey		Light Trap				
WV	38.26990N, 79.85243W	12 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.26990N, 79.85243W	12 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.26990N, 79.85243W	12 Jul 2006	L. Harvey		Light Trap				
WV	38.26990N, 79.85243W	12 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.46220N, 79.13544W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			

Table 1 (Continued). ***Dipectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
WV	38.36432N, 79.20688W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.32934N, 79.23275W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.33890N, 79.20690W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.70690N, 79.58665W	30 Jul 2006	L. Harvey		Stream Sample	XLarva			
WV	38.74146N, 79.68793W	30 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	37.92438N, 78.96567W	16 Jun 2005	D. Lenat		Stream Sample	4 larva			
VA	38.26950N, 79.30860W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	37.79108N, 79.70398W	14 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	37.79108N, 79.70398W	14 Jul 2006	L. Harvey		Light Trap				

Table 1 (Continued). ***Dipectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
VA	37.60295N, 80.07851W	15 Jul 2006	L. Harvey		Stream Sample	X Larva			
VA	37.60295N, 80.07851W	15 Jul 2006	L. Harvey		Light Trap				
VA	36.74647N, 81.42462W	26 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	36.74647N, 81.42462W	26 Jul 2006	L. Harvey		Light Trap				
VA	37.54145N, 79.58633W	27 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	37.56048N, 79.50323W	27 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	38.73822N, 78.53477W	28 Jul 2006	L. Harvey		Stream Sample	XLarva			
VA	37.92702N, 79.62438W	29 Jul 2006	L. Harvey		Stream Sample	XLarvaNC			
NC		17 Apr 2006	D. Lenat	NC5	Stream Sample	3 larva			

Table 1 (Continued). ***Dipectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
NC	35.0343N, 83.2359W		Highlands Class			3 larva	X		
NC			L. Harvey	BMT1		10 larva	X		
NC			L. Harvey	BMT2		4 larva	X		
OH	2 mile Creek	11 May 2005	M. Bolten	OH1	1 larva	X			
OH	Site 55	11 May 2005	M. Bolten	OH2	1 larva	X			
OH	Deleware. Co	11 May 2005	M. Bolten	OH3	1 larva	X			
OH	2 mile Creek	11 May 2005	M. Bolten	OH4	4 larva				
OH	2 mile Creek	11 May 2005	M. Bolten	OH5	5 larva				
OH	Site 55	11 May 2005	M. Bolten	OH6	3 larva				

Table 1 (Continued). ***Dipectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
NC		April 2006	D. Lenat	NC4	Stream Sample				
NC		6 Jun 2005	P. Phillips	NC2	Stream Sample	1 larva			
NC		6 Jun 2005	P. Phillips	NC3	Stream Sample	1 larva			
NC	34.01790N, 83.24510W		C. Geraci						
OH	site 55	01 Jun 2005	M. Bolten	OH7		2 larva			
OH	site 55	23 May 2005	M. Bolten	OH8		1 larva			
OH	del co	11 May 2005	M. Bolten	OH9		3 larva			
OH	del co	11 May 2005	M. Bolten	OH9		3 larva			
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC1	Stream Sample	1 larva	X		

Table 1 (Continued). ***Dipectrona*** **collected, examined, and sequenced.**

State	Latitude/longitude	Date	Collector	Code	Method	# of Specimens	COI	D2	Haplotype
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC2	Stream Sample	1 larva	X		
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC3	Stream Sample	1 larva	X		2
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC4	Stream Sample	1 larva	X		2
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC5	Stream Sample	1 larva	X		6
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC6	Stream Sample	1 larva	X		2
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC7	Stream Sample	1 larva	X		2
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC8	Stream Sample	1 larva			
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC9	Stream Sample	1 larva	X		2
SC	34.43885N, 82.18915W	16 Jun 2006	M. Biondi	SC10	Stream Sample	1 larva	X		2

KY	36.95509N, 84.39956W	15 Jun 2006	L. Harvey	KY1	Stream Sample	1 larva

Table 2. BOLD *Diplectrona modesta* collection data

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
HIEPT006-09	08-Jun-2009	*Diplectrona metaqui*	HPGP4	North Carolina State University, Centennial Campus
CJCAD054-07	26-Feb-2005	*Diplectrona metaqui*	HPGP4	Smithsonian Institution
OFDIP201-08	09-Jun-2002	*Diplectrona metaqui*	HPGP4	Smithsonian Institution, National Museum of Natural History
PKCAD370-07	01-Mar-2006	*Diplectrona metaqui*	HPGP4	University of Tennessee, Knoxville
PKCAD366-07	11-Apr-2006	*Diplectrona metaqui*	HPGP4	University of Tennessee, Knoxville
PKCAD310-07	31-May-2007	*Diplectrona modesta*	HPGP2	University of Tennessee, Knoxville
LHCAD017-07	09-May-2005	*Diplectrona modesta*	HPGP6	Clemson University
LHCAD016-07	06-Jun-2005	*Diplectrona modesta*	HPGP1	Clemson University
LHCAD014-07	11-May-2005	*Diplectrona modesta*	HPGP1	Clemson University
LHCAD013-07	16-Jun-2005	*Diplectrona modesta*	HPGP6	Clemson University
LHCAD011-07	16-Jun-2006	*Diplectrona modesta*	HPGP2	Clemson University
LHCAD010-07	16-Jun-2006	*Diplectrona modesta*	HPGP2	Clemson University
LHCAD012-07	15-Aug-2005	*Diplectrona modesta*	HPGP2	Clemson University
LHCAD015-07	23-May-2005	*Diplectrona modesta*	HPGP1	Clemson University
LHCAD019-07	28-Aug-2004	*Diplectrona modesta*	HPGP6	Clemson University
KKCAD231-07		*Diplectrona modesta*	HPGP2	Rutgers, The State University of New Jersey
HIEPT058-09	07-Jun-2009	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
HIEPT057-09	05-Jun-2009	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
HIEPT056-09	05-Jun-2009	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
HIEPT055-09	01-Jun-2009	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
HIEPT054-09	01-Jun-2009	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
HIEPT007-09	08-Jun-2009	*Diplectrona modesta*	HPGP1	North Carolina State University, Centennial Campus
FLCAD156-09	07-Aug-2008	*Diplectrona modesta*	HPGP6	University of Minnesota Insect Collection
FLCAD106-09	07-Aug-2008	*Diplectrona modesta*	HPGP6	University of Minnesota Insect Collection
FLCAD096-09	07-Aug-2008	*Diplectrona modesta*	HPGP6	University of Minnesota Insect Collection
CRCAD046-07	06-Jun-2007	*Diplectrona modesta*	HPGP6	Clemson University
CRCAD045-07	10-Jun-2007	*Diplectrona modesta*	HPGP6	Clemson University
CRCAD030-07	28-May-2007	*Diplectrona modesta*	HPGP6	Clemson University

Table 2. BOLD *Diplectrona modesta* collection data (continued)

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
CRCAD029-07	26-May-2007	*Diplectrona modesta*	HPGP1	Clemson University
OFDIP259-09	------	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP258-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP255-08	16-Jun-2008	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP235-08	30-May-1990	*Diplectrona modesta*	HPGP7	Smithsonian Institution, National Museum of Natural History
OFDIP234-08	30-May-1990	*Diplectrona modesta*	HPGP7	Smithsonian Institution, National Museum of Natural History
OFDIP233-08	30-May-1990	*Diplectrona modesta*	HPGP7	Smithsonian Institution, National Museum of Natural History
OFDIP230-08	30-May-1990	*Diplectrona modesta*	HPGP7	Smithsonian Institution, National Museum of Natural History
OFDIP229-08	30-May-1990	*Diplectrona modesta*	HPGP7	Smithsonian Institution, National Museum of Natural History
OFDIP228-08	24-Aug-2006	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP226-08	14-May-2003	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP225-08	14-May-2003	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP224-08	19-Oct-2003	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP223-08	20-Jul-2007	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP222-08	14-Jul-2006	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP221-08	24-Aug-2006	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP219-08	24-Aug-2006	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP217-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP216-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP214-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP212-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP211-08	09-Jun-2002	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP209-08	09-Jun-2002	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP208-08	14-May-2003	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP207-08	14-May-2003	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP205-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP204-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP203-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History

Table 2. BOLD *Diplectrona modesta* collection data (continued)

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
OFDIP202-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP199-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP198-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP197-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP195-08	09-Jun-2002	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP194-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP193-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP192-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP191-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP190-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP189-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP188-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP187-08	10-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP186-08	09-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP185-08	06-Jul-1987	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP184-08	06-Jul-1987	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP182-08	06-Jul-1987	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP181-08	06-Jul-1987	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP180-08	06-Jul-1987	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP179-08	11-Oct-2001	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP178-08	07-Jun-2001	*Diplectrona modesta*	HPGP2	Smithsonian Institution, National Museum of Natural History
OFDIP177-08	08-Jun-2002	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP174-08	30-Jun-1982	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP127-08	17-Jul-1964	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP126-08	28-Jul-1979	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP113-08	09-Jul-1982	*Diplectrona modesta*	HPGP6	Smithsonian Institution, National Museum of Natural History
OFDIP112-08	12-Jun-1979	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
PKCAD731-08	04-Jun-2008	*Diplectrona modesta*	HPGP1	University of Tennessee, Knoxville

Table 2. BOLD *Diplectrona modesta* collection data (continued)

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
PKCAD502-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD500-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD499-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD498-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD497-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD496-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD495-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD494-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD493-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD492-07	24-May-2006	Diplectrona modesta	HPGP6	Cowpens National Battlefield
PKCAD489-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD486-07	24-May-2006	Diplectrona modesta	HPGP6	Cowpens National Battlefield
PKCAD485-07	24-May-2006	Diplectrona modesta	HPGP2	Cowpens National Battlefield
PKCAD454-07	18-Jul-2007	Diplectrona modesta	HPGP1	Blue Ridge Parkway
PKCAD453-07	18-Jul-2007	Diplectrona modesta	HPGP6	Blue Ridge Parkway
PKCAD452-07	18-Jul-2007	Diplectrona modesta	HPGP1	Blue Ridge Parkway
PKCAD376-07	23-Feb-2006	Diplectrona modesta	HPGP2	University of Tennessee, Knoxville
PKCAD375-07	22-Feb-2006	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD374-07	23-Mar-2006	Diplectrona modesta	HPGP7	University of Tennessee, Knoxville
PKCAD369-07	23-Mar-2006	Diplectrona modesta	HPGP7	University of Tennessee, Knoxville
PKCAD347-07	11-May-2006	Diplectrona modesta	HPGP6	University of Tennessee, Knoxville
PKCAD346-07	04-Apr-2006	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD300-07	11-Jul-2007	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD252-07	04-Apr-2006	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD243-07	05-Apr-2006	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD151-07	22-Jun-2007	Diplectrona modesta	HPGP2	University of Tennessee, Knoxville
PKCAD146-07	18-Jul-2007	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD141-07	17-Jul-2007	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville

Table 2. BOLD *Diplectrona modesta* collection data (continued)

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
PKCAD140-07	21-Sep-2007	Diplectrona modesta	HPGP1	University of Tennessee, Knoxville
PKCAD127-07	15-Mar-2006	Diplectrona modesta	HPGP2	University of Tennessee, Knoxville
ONCAD006-07	30-May-2002	Diplectrona modesta	HPGP1	University of Waterloo
ONCAD005-07	30-May-2002	Diplectrona modesta	HPGP1	University of Waterloo
ONCAD004-07	30-May-2002	Diplectrona modesta	HPGP1	University of Waterloo
ONCAD003-07	30-May-2002	Diplectrona modesta	HPGP1	University of Waterloo
ONCAD002-07	30-May-2002	Diplectrona modesta	HPGP1	University of Waterloo
LHCAD006-07	16-Jun-2006	Diplectrona modesta	HPGP6	Clemson University
LHCAD008-07	16-Jun-2006	Diplectrona modesta	HPGP2	Clemson University
LHCAD007-07	16-Jun-2006	Diplectrona modesta	HPGP2	Clemson University
LHCAD005-07	16-Jun-2006	Diplectrona modesta	HPGP2	Clemson University
LHCAD004-07	16-Jun-2006	Diplectrona modesta	HPGP2	Clemson University
LHCAD003-07	06-Sep-2005	Diplectrona modesta	HPGP6	Clemson University
LHCAD002-07	06-Sep-2005	Diplectrona modesta	HPGP6	Clemson University
LHCAD001-07	30-May-2005	Diplectrona modesta	HPGP1	Clemson University
LHCAD020-07	01-Jun-2005	Diplectrona modesta	HPGP1	Clemson University
LHCAD018-07	28-Aug-2004	Diplectrona modesta	HPGP6	Clemson University
OFDIP111-08	30-May-1990	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP097-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP096-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP095-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP094-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP093-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP092-08	17-Jun-1992	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP090-08	24-Aug-1983	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP089-08	24-Aug-1983	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP088-08	24-Aug-1983	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP080-08	24-Aug-1983	Diplectrona modesta	HPGP1	Smithsonian Institution, National Museum of Natural History

Table 2. BOLD *Diplectrona modesta* collection data (continued)

Sequence Code	Collection Date	Species	Haplotype	Voucher Location
OFDIP069-08	24-Aug-1983	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP068-08	24-Aug-1983	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP062-08	21-Jun-1986	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP060-08	21-Jun-1986	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP029-08	09-Aug-1986	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
OFDIP009-08	17-May-1970	*Diplectrona modesta*	HPGP1	Smithsonian Institution, National Museum of Natural History
ECCAC059-09	18-Aug-2009	*Diplectrona modesta*	HPGP1	Biodiversity Institute of Ontario
ECCAC051-09	18-Aug-2009	*Diplectrona modesta*	HPGP1	Biodiversity Institute of Ontario
ECCAC043-09	18-Aug-2009	*Diplectrona modesta*	HPGP1	Biodiversity Institute of Ontario
ECCAC003-09	18-Aug-2009	*Diplectrona modesta*	HPGP1	Biodiversity Institute of Ontario
ECCAC526-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC514-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC512-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC504-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC502-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC469-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC418-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC404-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC403-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC402-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC385-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC384-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC383-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC378-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC375-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC370-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC369-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC367-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario

Table 2. BOLD *Diplectrona modesta* collection data (continued)

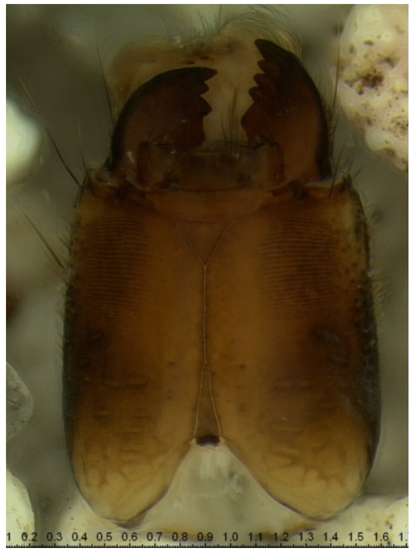
Sequence Code	Collection Date	Species	Haplotype	Voucher Location
ECCAC364-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC361-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC360-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC358-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC356-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC350-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC348-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC328-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC324-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC304-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC301-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC296-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC295-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC294-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC290-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC288-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario
ECCAC286-09	18-Aug-2009	*Diplectrona modesta*	HPGP2	Biodiversity Institute of Ontario

A.  B. 

C. 

Figure 1.1. *Diplectrona modesta* Haplotype 1. Specimen from Great Smokey Mountains National Park, TN. A. Dorsal view of head capsule. B. Ventral view of head capsule. C. Left lateral view of head.


.A.


B.


C.

Figure 1,2. *Diplectrona modesta* Haplotype 2. Specimen from Baldwin Creek, Greenville, County, South Carolina. A. Dorsal view of head capsule. B. Ventral view of head capsule. C. Left lateral view of head capsule.

A.

B.

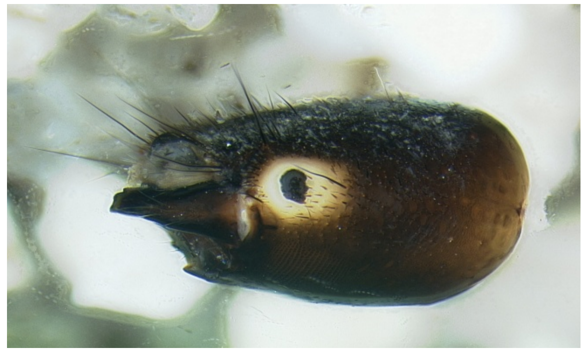
Figure 1.3. *Diplectrona metaqui* Haplotype 4. Specimen from unnamed tributarys, Oconee County, South Carolina. A. Dorsal view of head capsule. B. Left lateral view of head capsule, showing diagnostic mandibular “thumb”.

A. 

B. 

C. 

Figure 1.4. *Diplectrona modesta* Haplotype 6. Specimen from Blue Ridge Parkway, Augusta County, Virginia. A. Dorsal view of head capsule. B. Ventral view of head capsule. C. Left lateral view of head capsule.

A.  B. 

C. 

Figure 1.5. *Diplectrona modesta* Haplotype 7. Specimen collected from Great Smokey Mountains National Park, TN. A. Dorsal view of head capsule. B. Left lateral view of head capsule. Left lateral view of body.

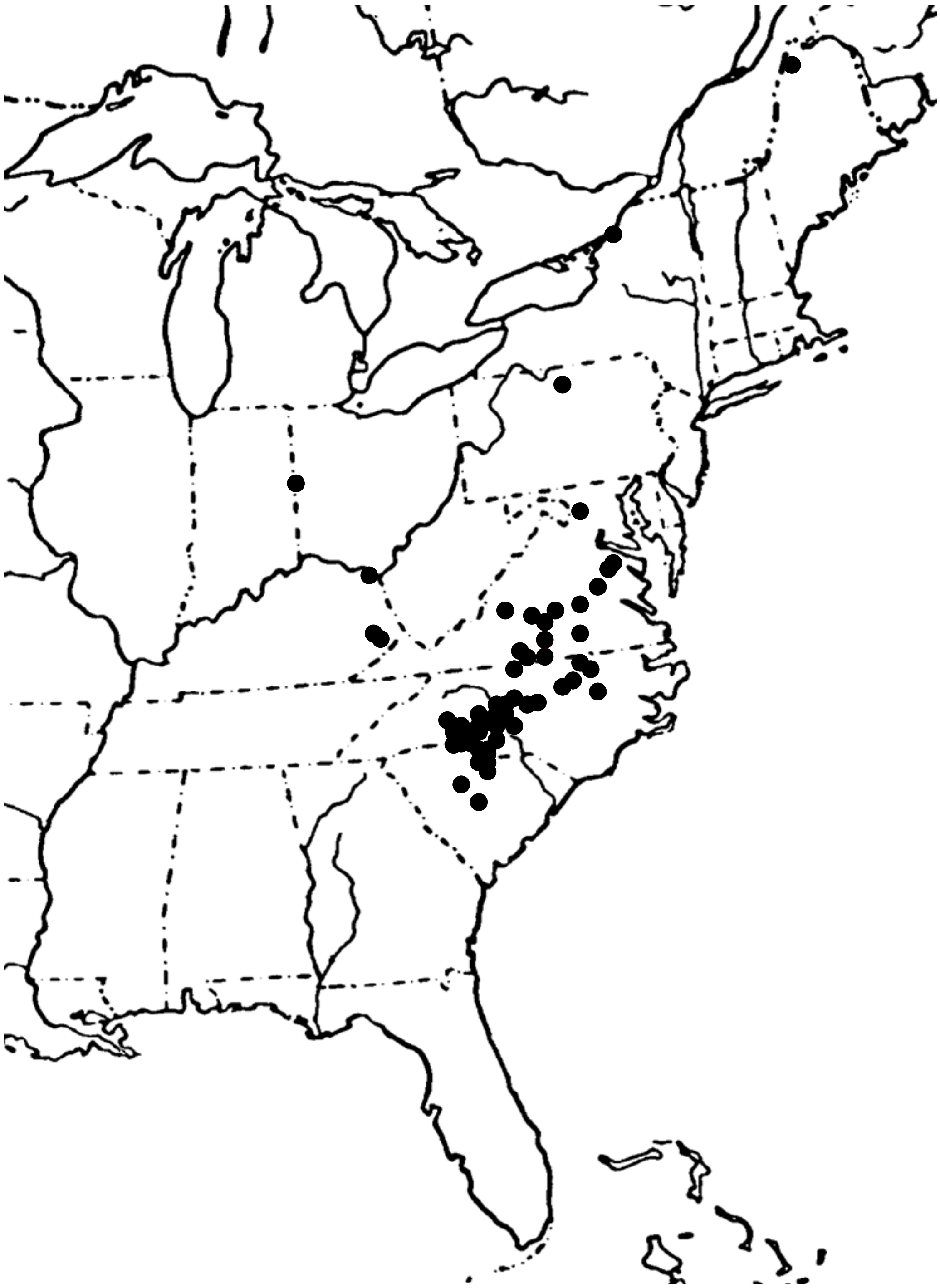


Figure 2.1. *Diplectrona modesta Banks* collection sites and BOLD sites for Haplotypes 1 and 4.

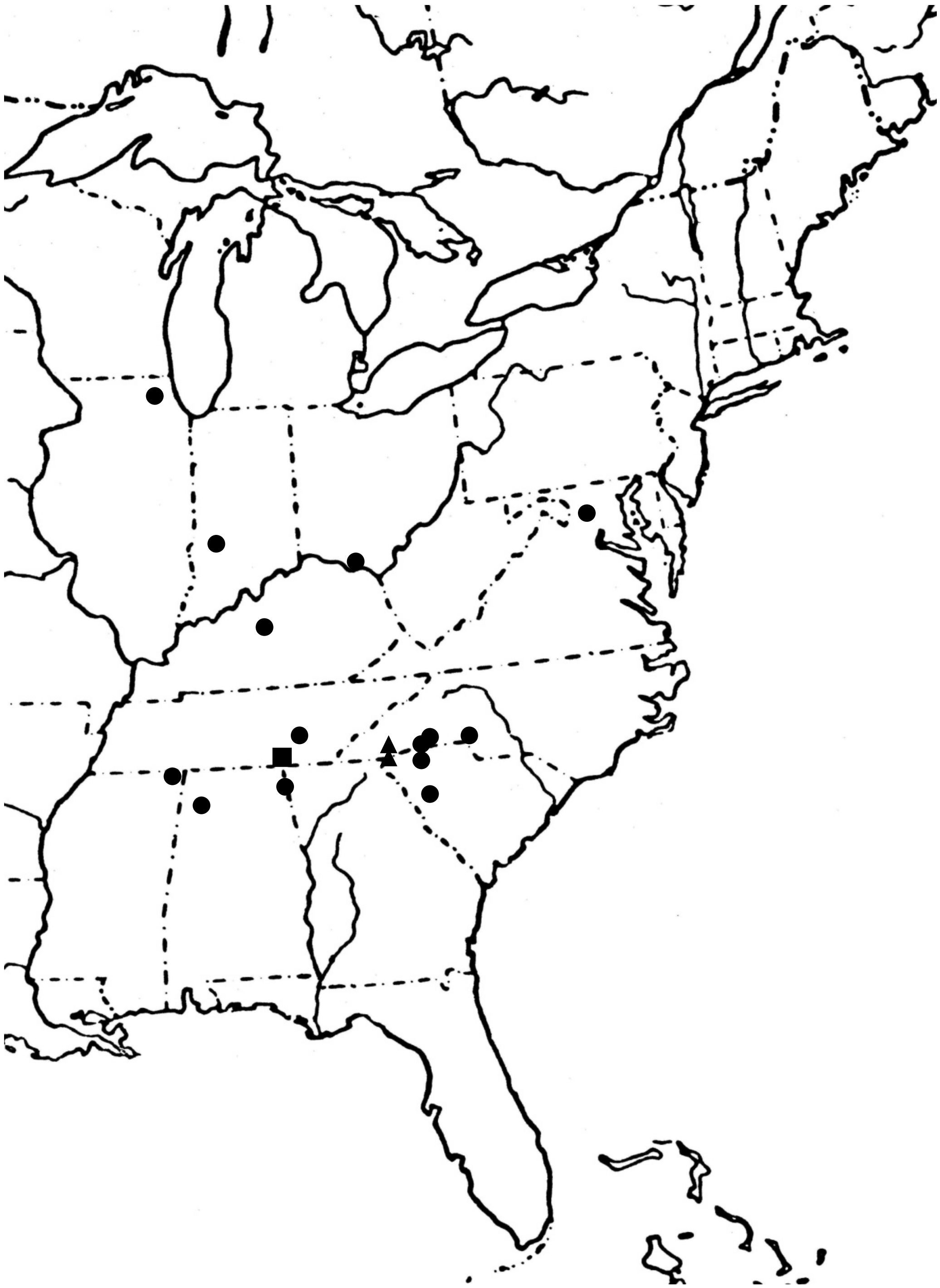


Figure 2.2. *Diplectrona modesta* Banks collection sites. Haplotype 2 (●). Haplotype 3 (▲). Haplotype 5 (■).

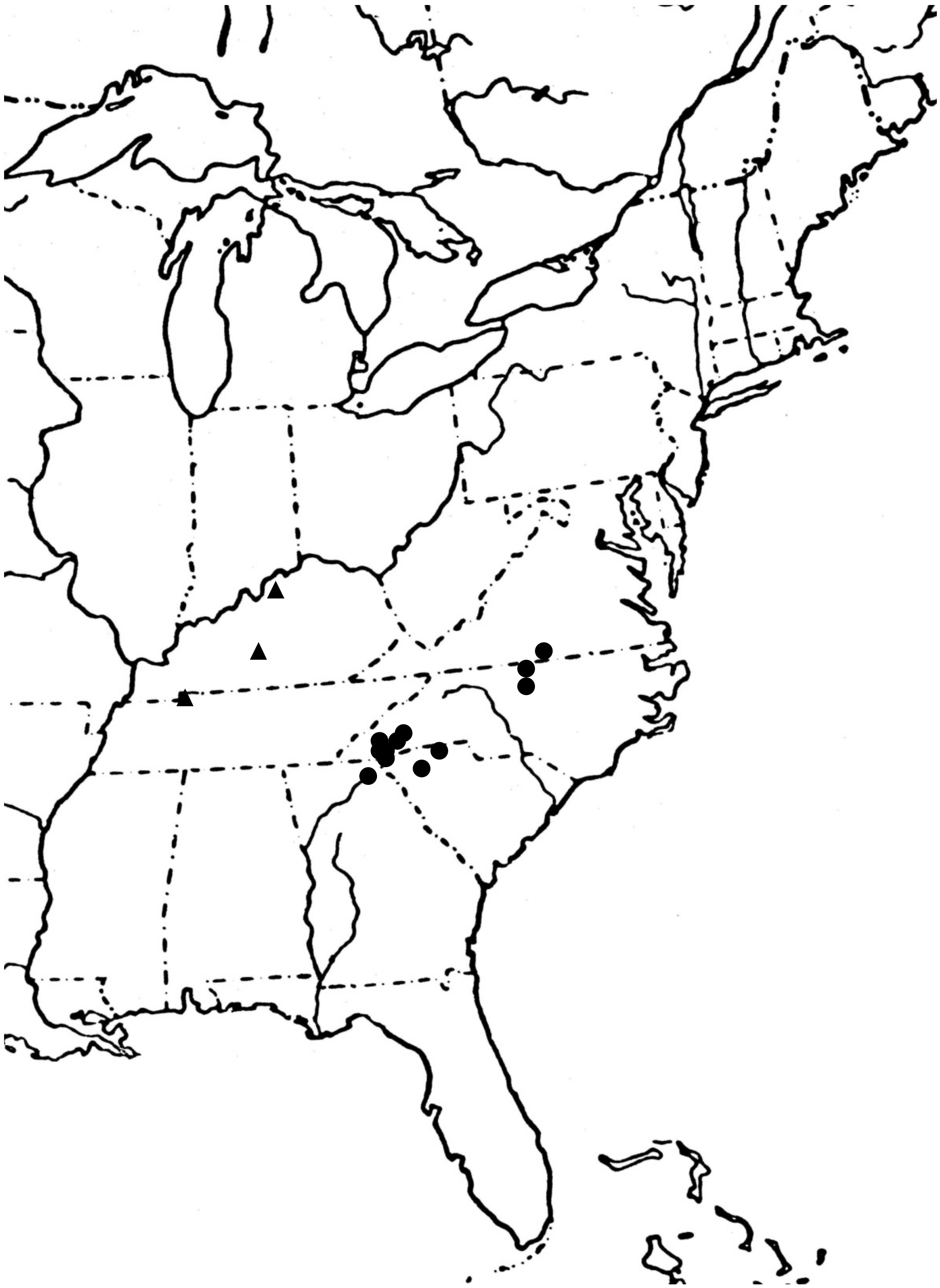


Figure 2.3. *Diplectrona modesta* Banks collection sites. Haplotype 6 (●). Haplotype 7 (▲).

Figure 3.1. Neighbor-joining tree of COI gene in LHCAD *Diplectrona modesta* samples.

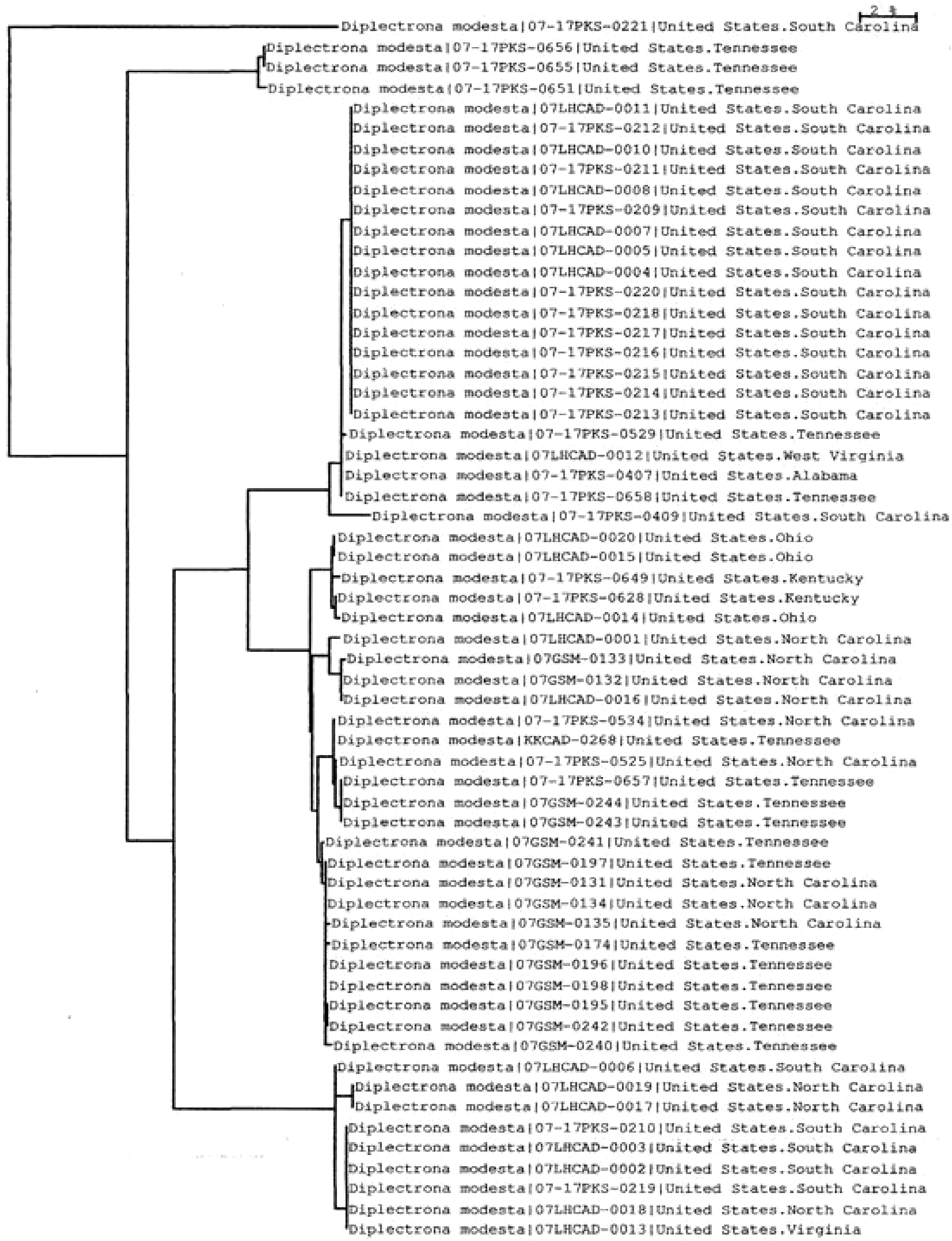


Figure 3.1. Diplectrona modesta, neighbor-joining tree using BOLD COI sequences.

Figure 3.1. Diplectrona modesta, neighbor-joining tree using BOLD COI sequences.

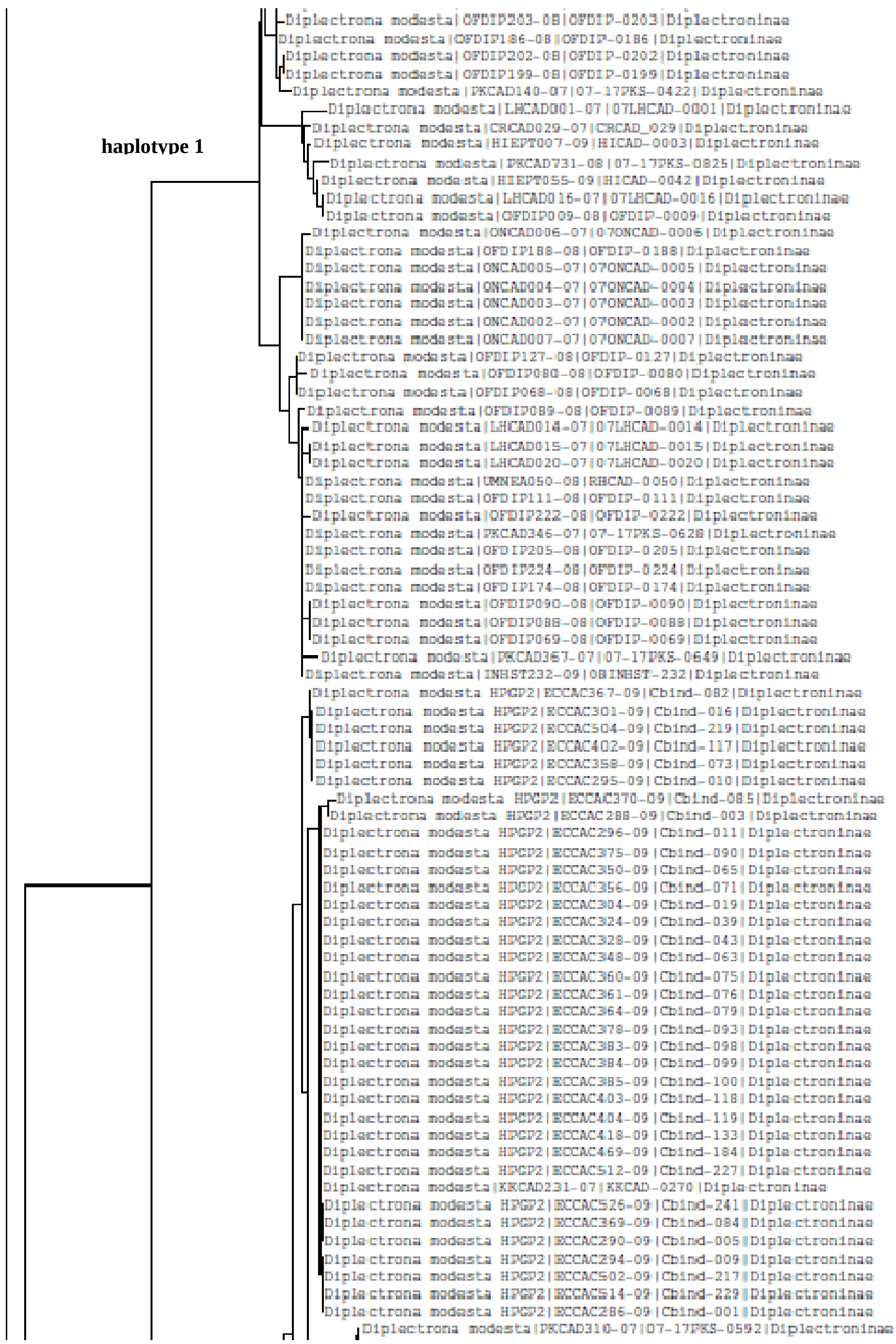


Figure 3.1. Diplectrona modesta, neighbor-joining tree using BOLD COI sequences (continued).

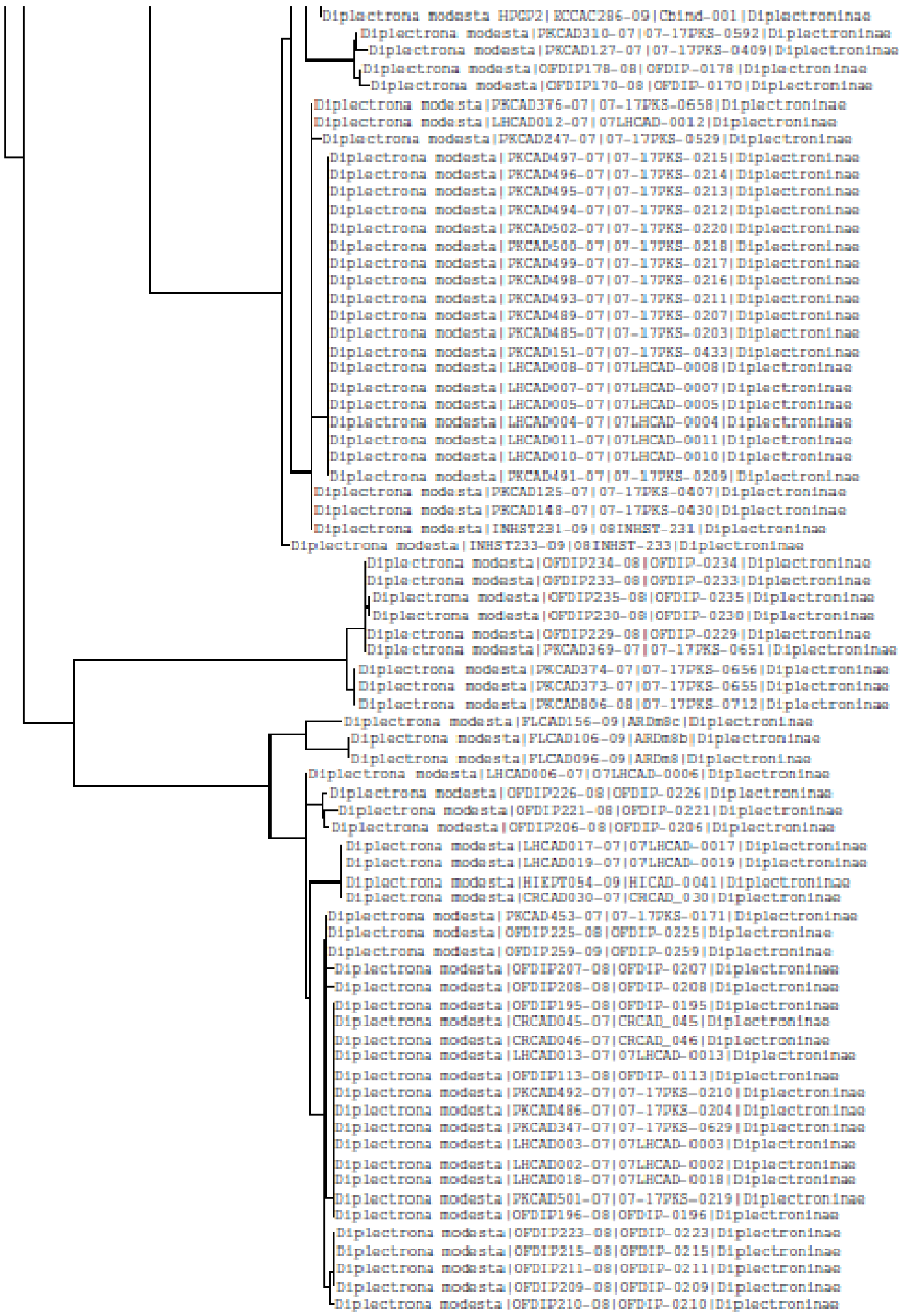
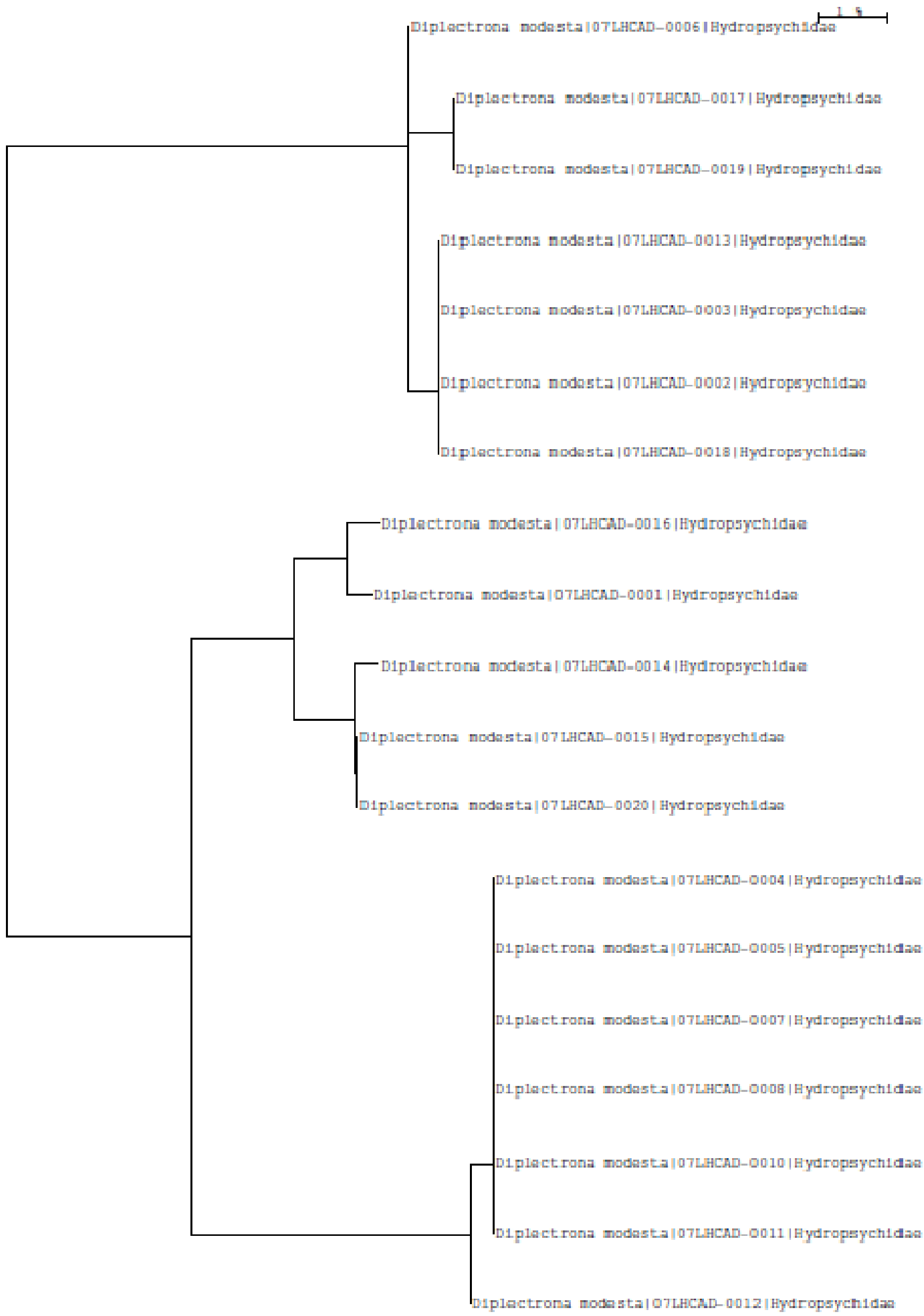


Figure 3.2. LHCAD neighbor-joining tree.



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